Amendments to the Claims

Claim 1 (amended)

An assay for trypsin inhibitors in urine which comprises contacting a urine test sample with a buffered assay medium comprising trypsin, a substrate for trypsin which will produce a detectable response when cleaved by trypsin and a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine as assay reagents and correlating the concentration of trypsin inhibitor with the detectable response from the cleaving of the substrate.

Claim 5 - Change "the" to ---a--- in line 2 of this claim.

Remarks

Applicants confirm the provisional election of Group 1 (Claims 1-10) with traverse. Contrary to the Examiner's position, Claims 1-10 which cover an assay for trypsin inhibitors and claims 11-14 which cover a method for preparing a test device, which has no other use than to carry out the assay of Claims 1-10, are not unrelated inventions and are not separate and distinct inventions. Accordingly, the restriction requirement is unwarranted and it is submitted that it should be withdrawn.

Claim 2 has been rejected under 35 USC 112 as lacking antecedent basis for "the assay reagents". This ground of rejection has been obviated by the foregoing amendment to Claim 1.

Claims 3 and 4 have been rejected under 35 USC 112 as lacking antecedent basis for "the solvent". This rejection is

believed to be unwarranted because Claim 3 depends on Claim 2 which includes the term solution. This adds inherent antecedent basis for "solvent" in Claim 3 because a solution must have a solvent.

In Claim 5 "the dry phase" has been changed to "a" dry phase to obviate the rejection of this claim.

Claims 1-4 and 7-9 have been rejected under 35 USC 103(a) as being rendered obvious by Uenoyama et al. in view of Berry et al. The Examiner points out that Uenoyama et al. disclose a method for measuring the concentration of urinary trypsin inhibitors by mixing the urine sample, trypsin and a buffer solution followed by adding a substrate solution to cause the enzyme reaction and measuring the activity of trypsin. The Examiner notes that this reference differs from the present claims by failing to disclose the use of polycarboxylic chelating agents to inhibit calcium interference but points out that Berry et al. teach the use of EGTA and EDTA as chelating agents which inhibit the interfering ions of calcium in urine samples.

The foregoing ground of rejection is believed to be unwarranted. It should be noted that the method of measuring trypsin inhibitors disclosed by Uenoyama et al. involves the addition of a buffer which contains at least 0.15 μmol of calcium per μg of the added trypsin. The purpose of using excess calcium is to reduce the interference of calcium salts already in the urine sample by adding additional calcium to swamp out its effect. In the present invention, the addition of additional calcium is not necessary and is even detrimental since in the method of Uenoyama et al. the calcium interference is not removed but only offset.

Berry et al. disclose a method which is specific for sodium and uses a sodium sensitive enzyme which is interfered with by calcium. This method differs from the present invention in that it uses a sodium sensitive enzyme and a selective binding agent which binds to these ions, e.g. sodium, the concentration of which is being determined. Furthermore:

- Sodium is not an interferant but is the analyte being measured. There is no mention of a selective binding agent which selectively binds to an interferant and Berry et al. would not lead one skilled in the art to believe that a selective binding agent could be used to remove an interferant such as calcium.

- The effect of the ions which are chelated in Berry et al. is specific to the enzyme (they mention transferase, hydrolase, oxidoreductase and lyase) whereas trypsin is a protease and is insensitive to monovalent cations like sodium with neutral substrates.

- Most enzymes are sensitive to selective binding agents such as chelating agents and it is unexpected that trypsin is not affected by these selective binding agents. The mechanisms vary greatly between enzymes and substrates. In fact, the charging of a counter anion, as the chelating agent would do, increases not decreases the inhibition of trypsin by monovalent salts.

Accordingly, it would not have been obvious to one skilled in the art of using enzymes that a chelating agent such as EGTA would prevent inhibition of trypsin by calcium ion without a reduction in enzyme activity. However, as applicants have demonstrated in the examples set out in the present application, the presence of EGTA in the trypsin inhibitor assay results in reduced variation in the assay without loss in trypsin activity.

Furthermore, there is no suggestion in either reference that chelating agents could, or should, be used in assays for trypsin inhibitors in which trypsin is the enzyme. The combination of Uenoyama et al. who use excess calcium ion to partially drown out

the calcium interference and Berry et al., who use binding agents in assays for certain ions (particularly sodium) which stimulate certain enzymes (other than trypsin) do not suggest to one skilled in this art that a chelating agent would be effective to reduce calcium interference in an assay for trypsin inhibitors in urine to provide a test with significantly reduced variation.

Claims 5 and 6 have been rejected under 35 USC 103(a) as being rendered obvious by Uenoyama et al. in view of Berry et al. as applied above and further in view of GB 2,204,398 (May et al.). May et al. is cited for its teaching of a dry test device into which the reagents are incorporated and the Examiner concludes that it would have been obvious to use the method of Uenoyama et al. as modified by Berry et al. in the strip of May et al. This ground of rejection is also traversed because, as established above, Uenoyama et al. and Berry et al. do not render obvious the trypsin inhibitor assay of the present invention in the wet form and the inclusion of May et al. does not render it obvious in the dry form. The invention of the present claims 5 and 6 provides a convenient method for the determination of trypsin inhibitors by use of a dry test system containing trypsin and a substrate for trypsin which provides a detectable response when cleaved by trypsin. A chelating agent is included in the assay to reduce interference caused by calcium present in the urine test sample. As has been established, this assay is not rendered obvious by the prior art and it is even more unobvious that the assay could be used in the dry form.

Claim 10 has been rejected under 35 USC 103(a) as being unpatentable over Uenoyama et al. in view of Berry et al. as applied to Claims 1-4 and 7-9 and further in view of Nanbu et al. for this reference's disclosure of arginine and lysine derivatives as substrates for trypsin. While the substrate for trypsin may be disclosed by Nanbu et al., Claim 10 depends on

Claim 1 which has been shown to be novel and unobvious. Accordingly, Claim 10, which adds a further limitation to Claim 1, is not rendered obvious by the combination of references.

Conclusion

The various rejections have either been obviated by amendment or shown to have been unwarranted. Reconsideration of these rejections and allowance of the claims pursuant to such reconsideration are requested.

Respectfully submitted,

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APPENDIX A

Claim 1 (amended)

An assay for trypsin inhibitors in urine which comprises contacting a urine test sample with a buffered assay medium comprising trypsin, a substrate for trypsin which will produce a detectable response when cleaved by trypsin and a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine as assay reagents and correlating the concentration of trypsin inhibitor with the detectable response from the cleaving of the substrate.

Claim 5 (Amended)

The assay of Claim 1 wherein the assay reagents are in a dry phase.